Seasonal effects on the corneoconjunctival microflora in a population of Persian cats in Iran

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Abstract: This study was performed to determine the normal seasonal aerobic and an-aerobic corneoconjunctival bacterial flora in cats. Thirty eyes of 15 clinically normal client-owned Persian cats were evaluated. All cats lived in a similar indoor/outdoor home environment being fed the same diet for the entire year. The cats did not receive any medications and were found to be clinically healthy 1 week prior to each microbial sampling. The cats were not exposed to other cats during the study period. Microbial samples were collected at the same time of day on the first day of the second month of each of the four seasons. During sample collection, a sterile swab was rolled over the corneoconjunctival surface avoiding contact with surrounding skin or hair. Immediately after sample collection, microbiologic aerobic and anaerobic cultures were initiated. Gram-positive bacteria were the most prevalent isolates. The most commonly isolated bacterial organisms across all seasons were Staphylococcus epidermidis (41/95; 43.2%), -hemolytic streptococcus (18/95; 18.9%), Staphylococcus aureus (17/95; 17.9%), and Escherichia coli (11/95; 11.5%). Twenty-five cultures of a total of 120 (20.8%) were negative. One negative culture was collected in the summer, while 21 cultures were negative in fall and winter. Gram-positive bacteria were the predominant micro-organisms of the normal ocular surface of healthy cats in all seasons in this study. This result is in agreement with previous publications.

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Short Communication

Seasonal Effects on the Corneoconjunctival Microflora in a Population of Persian Cats in Iran

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A B S T R A C T

This study was performed to determine the normal seasonal aerobic and an-aerobic corneoconjunctival bacterial flora in cats. Thirty eyes of 15 clinically normal client-owned Persian cats were evaluated. All cats lived in a similar indoor/outdoor home environment being fed the same diet for the entire year. The cats did not receive any medications and were found to be clinically healthy 1 week prior to each microbial sampling. The cats were not exposed to other cats during the study period. Microbial samples were collected at the same time of day on the first day of the second month of each of the four seasons. During sample collection, a sterile swab was rolled over the corneoconjunctival surface avoiding contact with surrounding skin or hair. Immediately after sample collection, microbiologic aerobic and anaerobic cultures were initiated. Gram-positive bacteria were the most prevalent isolates. The most commonly isolated bacterial organisms across all seasons were *Staphylococcus epidermidis* (41/95; 43.2%), β-hemolytic streptococcus (18/95; 18.9%), *Staphylococcus aureus* (17/95; 17.9%), and *Escherichia coli* (11/95; 11.5%). Twenty-five cultures of a total of 120 (20.8%) were negative. One negative culture was collected in the summer, while 21 cultures were negative in fall and winter. Gram-positive bacteria were the predominant micro-organisms of the normal ocular surface of healthy cats in all seasons in this study. This result is in agreement with previous publications.

Introduction

The normal microflora of the ocular surface refers to nonpathogenic resident aerobic and anaerobic bacteria found on healthy ocular surfaces such as conjunctiva and cornea.[1,2] These micro-organisms may originate from the skin and colonize the ocular surfaces.[3] This constitutes a stable ecosystem unless disrupted by diseases and disorders of the ocular surface, trauma, surgery or the use of antibiotics.[3] The corneoconjunctival microflora of dogs, cats, and horses has been evaluated in health and disease.[4–13] The presence of different micro-organisms on the ocular surface depends on the age, environment, season, geography, host defenses, husbandry, and bedding in different animal species.[2–4]

The effect of seasonal factors has been evaluated in the horse.[2] Factors such as humidity and temperature have a major impact on the conjunctival microbial flora in horses.[2,14] To the best of the authors' knowledge, this study is the first report on the seasonality of the normal corneoconjunctival bacterial flora of clinically normal cats. The aim of this study was to determine the potential seasonal effects on the microflora of the normal Persian cat corneoconjunctival surface in Tehran, Iran.

Materials and Methods

The present study was approved by the Iran Society for Prevention of Cruelty to Animals following the Iranian ethical codes for studies on laboratory animals, and conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Fifteen clinically normal adult Persian cats were used in this study. Inclusion criteria were (1) healthy cats with normal physical and ophthalmic examination; (2) normal complete blood count and biochemistry profile; (3) negative polymerase chain reaction test for *Mycoplasma* spp., *chlamydophila* spp., and feline herpes virus-1; (4) living in/outdoors with constant availability of food; (5) single animal household. Exclusion criteria were (1) cats younger than 12 months; (2) cats with systemic or ocular diseases; and (3) cats with unilateral or bilateral nasolacrimal duct obstruction.

Microbiological samples were obtained at the same time of day on the first day of the second month of each season (April, July, October, and January). Tehran (Teheran) has a predominantly dry climate, being geographically located between mountains and desert. The average temperature in April, July, October, and January is 20°C, 35°C, 25°C, and 6°C, respectively.[15] All cats lived in a normal single animal home environment with access to a fenced-in outdoor area, access to water ad libitum, and were fed with the same commercial diet throughout the study. They had no contact with other cats or animals during the study period.

Full physical and ophthalmic examinations were performed 7 days before sampling in each season to ensure that all cats were healthy. Ocular examinations and sampling were performed by a single examiner and culture and microbiological test were performed by a single microbiologist.

Bacterial culture samples were obtained from both eyes in a random order with gentle physical restraint 15-30 seconds after applying 1 drop of topical anesthetic (Minims, 0.4% oxybuprocaine hydrochloride, Bausch & Lomb UK Ltd, Surrey, England). For the collection of samples, a sterile swab applicator was rolled over the corneal surface and mucosal surface of the ventral conjunctival fornix while avoiding contact with the surrounding skin or hair. Cultures were initiated immediately after sample collection. One MacConkey and 1 blood agar plate were incubated in an aerobic environment and the remaining plates in an anaerobic environment with 5% CO_2 present at 37°C for 24-48 hours. Examination of cultures was conducted

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and cultures were subcultured if needed after 24 hours. Individual colonies were analyzed using standard biochemical and microbiological procedures to identify bacterial genus/species (Gram stain, cellular morphology, colony characteristics, catalase, and oxidase reaction) as described by Quinn.[16] All animals were evaluated for the presence of *Mycoplasma* spp., *chlamydophila* spp., and Feline herpes virus-1 with polymerase chain reaction based on the method described by Vogtlin et al and Pinard et al prior to each sample collection.[17,18] Statistical analysis was performed using SPSS 20.0 (SPSS Inc, Chicago, IL). The data were evaluated via descriptive statistics. A chi-squared test for goodness-of-fit was performed to compare total numbers of isolated bacteria between seasons.

Results

Mean ± standard deviation age of animals was 2.8 ± 0.9 years. The age range was 1-4 years. Nine male and 6 female cats were included. All cats were neutered and castrated, respectively. Positive culture results were obtained in 95 of 120 (79.1%) samples. Of these positive samples, 84 of 95 (88.4%) isolates were Gram-positive bacteria. Anaerobic bacterial cultures were negative in all cats.

The most commonly isolated bacterial organisms across all seasons were *Staphylococcus epidermidis* (*S epidermidis;* 41/95; 43.2%), β-hemolytic streptococcus (18/95; 18.9%), and *Staphylococcus aureus* (17/95; 17.9%). Other isolated bacteria were *Escherichia coli* (*E coli;* 11/95; 11.5%), *Corynebacterium* spp. (5/95; 5.2%), and unspecified *Streptococcus* spp. (3/95; 3.1%). One negative culture was collected in the summer, while 21 cultures were negative in fall and winter. Although not statistically significant, fewer negative cultures were collected in the spring and summer than in the fall and winter. The chi-squared test for goodness-of-fit demonstrated no statistically significant difference in total numbers of isolated bacteria between seasons ($X^2 = 3.53$; df = 3; $P = .3$). Detailed results are presented in Table 1.

Discussion

This study demonstrated the dominance of Gram-positive species in the conjunctival bacterial flora of normal Persian cats, which was in agreement with data previously reported for cats and other species.[4-7,10-12,19-31] Three studies reported on the normal conjunctival flora of cats in Canada, Poland, and Brazil.[7,9,12] In these 3 studies, Gram-positive bacteria and specifically *S epidermidis* were reported as dominant conjunctival bacterial flora in cats.[7,9,12]

Our study failed to demonstrate a correlation between season and bacterial culture results in Persian cats living in the same indoor/outdoor home environment throughout the year. Although not statistically significant, an interesting finding in the present study was the decrease in overall number of positive bacterial cultures and number of Gram-negative bacteria isolates in the cold seasons as compared to hot seasons. *E coli* was the only type of Gram-negative bacteria isolated in this study and 6 of 11 isolates were collected in the spring. All 5 *Corynebacterium* spp. isolates were collected in the spring and summer. Twenty-one of 25 negative aerobic cultures were obtained in fall and winter and only 1 negative aerobic culture was collected in the summer. These results are suggestive of a seasonal influence on culture results but the number of samples and isolates is too small for solid conclusions.

Effects of seasonality on the conjunctival flora were studied in horses in Urmia in the northwest of Iran.[32] The authors investigated the conjunctival bacterial flora of 21 horses in all 4 seasons through 2011 and 2012. *Bacillus cereus*, *S epidermidis*, *Klebsiella oxytoca*, beta-hemolytic streptococci, and *E coli* were the most commonly isolated bacteria throughout the seasons in these Iranian horses. Fifty-nine percent of all isolates were Gram-positive bacteria and seasonality seemed to influence isolation rates of certain bacterial isolates.[32]

Andrews et al also reported effects of season on the conjunctival flora in horses in Florida.[2] In their study, *Corynebacterium* spp., *Staphylococcus* spp., *Bacillus* spp., and *Moraxella* spp. were the most frequently isolated bacteria. Gram-positive bacteria were the most commonly isolated bacteria with isolation rates that were evenly distributed across seasons. Isolation rates of Gram-negative bacteria were highest in the fall.[2]

Seasonal influences were also reported by Rubio et al who observed an increased rate of positive bacterial cultures during the months of April, May, and June collected from the conjunctival surface of 4432 human patients undergoing cataract surgery in Madrid, Spain.[33]

Grosas et al demonstrated that there was a huge difference in bacterial isolates collected from calf eyes at the end of the indoor housing (winter) season compared to the outdoor housing pasture (summer) season.[34] Which obviously implies a large influence of environment on the culture results. Cats of our study lived in the same indoor/outdoor environment throughout the year, which fits with a pretty stable distribution of isolate types. The cats moved in the same environment throughout the year whereas calves were indoor in the winter months and outdoor in the summer months, which clearly influenced the isolation rates of the *staphylococcus* spp. in compare to *Moraxella* bacteria.[34]

Considering that positive fungal cultures have been reported in 40% of cats in one study,[4] the first limitation of this study was the fact that fungal cultures were not performed. Second, limited sample size makes inferences regarding seasonal influence on isolation rates of single isolate types impossible. Third, the homogeneous population (all single-cat household cats of 1 breed in 1 geographic location) makes it difficult to extrapolate study results to other cat populations in different geographic locations or home environments (indoor vs. outdoor, single vs. multicat households).

In conclusion, this study revealed Gram-positive bacteria to be the most prevalent bacteria on the normal ocular surface of healthy Persian cats with no significant seasonal influence on bacterial isolation rates.

Table 1
Genera Cultured and Frequency of Isolation by Season of Bacteria Cultured From 120 Samples Obtained From the Corneal Surface and Conjunctival Sac of 30 Eyes From15 Persian Cats

Bacteria/Season	Spring	Summer	Fall	Winter
Staphylococcus epidermidis	9	13	10	9
Staphylococcus aureus	5	5	3	4
Corynebacterium spp.	2	3	0	0
β-hemolytic streptococcus spp.	5	4	4	5
Streptococcus spp.	0	1	1	1
Escherichia coli	6	3	2	0
Total	27	29	20	19

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.tcam.2018.12.003.

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